

Characterization of Feline *Helicobacter pylori* Strains and Associated Gastritis in a Colony of Domestic Cats

LAURENCE K. HANDT,^{1†} JAMES G. FOX,^{2*} ILSE H. STALIS,^{3‡} ROSEMARIE RUFO,¹ GWEN LEE,¹
JEFFREY LINN,¹ XIANTANG LI,² AND HARRY KLEANTHOUS⁴

University Laboratory Animal Resources¹ and Department of Veterinary Pathobiology, School of Veterinary Medicine,³
University of Pennsylvania, Philadelphia, Pennsylvania, 19104, and Division of Comparative Medicine,
Massachusetts Institute of Technology,² and Oravax,⁴ Cambridge, Massachusetts, 02139

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Twenty-four young adult domestic cats from a commercial vendor were found to be infected with *Helicobacter pylori*. Histopathologic analyses, selected electron microscopy, and urease mapping were performed on mucosal samples collected from the cardias and fundi, bodies, and antra of these cats' stomachs. *H. pylori* organisms were abundant in all areas of the stomach on the basis of histologic evaluation and urease mapping. *H. pylori* infection was associated with a moderate to severe lymphofollicular gastritis in 21 of 24 cats (88%). The gastritis was most pronounced in the antral region and consisted mainly of multifocal lymphoplasmacytic follicular infiltrates in the deep mucosa. The severity of gastritis in the antrum corresponded to high numbers of *H. pylori* there on the basis of the use of the urease assay as an indicator of *H. pylori* colonization. Ten of 24 cats (42%) also had small to moderate numbers of eosinophils in the gastric mucosa. All 24 cats had gastric lymphoid follicles, with follicles being most prevalent in the antrum. Electron microscopy of gastric tissue revealed numerous *H. pylori* organisms, some of which were closely adhered to the mucosal epithelium. Human *H. pylori* gene-specific primers to *ureA* and *ureB* amplified products of similar sizes from *H. pylori* cat isolates. Digestion of the products with restriction enzymes resulted in fragments characteristic of the restriction fragment length polymorphism patterns of *H. pylori* isolates from humans. In the domestic cat, *H. pylori* infection is associated with a lymphofollicular gastritis, consisting of lymphocytic and plasmacytic infiltration into the lamina propria, and the organism appears to provide chronic antigenic stimulation resulting in the formation of gastric lymphoid follicles.

Since the initial isolation of *Helicobacter pylori* from human gastric tissue in 1983, evidence has accumulated implicating the bacterium as a human pathogen (37). *H. pylori* is now known to be the causative agent of chronic active gastritis and duodenal ulcer (22, 23, 37) as well as a likely cofactor in the development of gastric adenocarcinoma in humans (17, 49). More recently, an association has been established between *H. pylori* and gastric MALT lymphoma (9, 29, 61).

Animals also have been noted to harbor *Helicobacter* spp. in inflamed gastric tissue. *Helicobacter mustelae* causes chronic gastritis in ferrets (16), and a group of cheetahs affected with severe lymphoplasmacytic gastritis harbored gastric *Helicobacter*-like organisms, as well as a previously unreported gastric spiral organism, *Helicobacter acinonyx* (11, 12). Spiral organisms have been observed in domestic feline and canine gastric tissues since the turn of the century (4). These gastric bacteria have received renewed attention because of the discovery of *H. pylori* and its role in gastroduodenal disease in humans. In 1987, a large spiral bacterium with periplasmic fibers was isolated from the gastric tissue of a cat and was named *Helicobacter felis* (39, 50). Another organism with a similar appearance, but lacking periplasmic fibers, has also been observed in feline and canine gastric tissues (39). This organism has not yet

been isolated, but it has been called the “*Gastrospirillum*-like organism.” Because *H. felis* and *Gastrospirillum*-like organisms cannot be distinguished when they are examined in gastric tissue by light microscopy (39), they are collectively referred to as “gastric *Helicobacter*-like organisms” (GHLOs). In early reports, these large gastric spirilla were considered to be innocuous commensal organisms in cats and dogs (28, 59). Recently, GHLOs were reported in 97% of adult cats studied, in association with inflamed gastric tissue (48). Other studies in cats have also documented the association of these organisms with chronic gastritis (14, 21).

Zoonotic transmission of gastric spiral organisms from domestic dogs and cats has been postulated, because bacteria with morphologies similar to those of both *H. felis* and other GHLOs have been observed in the stomachs of humans with gastritis (27, 34, 36, 39, 45, 57, 60). However, because domestic animals had never previously been found to harbor *H. pylori* naturally, zoonotic transmission had not been considered a significant risk factor in the epidemiology of *H. pylori* infection in humans. Our recent discovery of a colony of domestic felines infected with *H. pylori* not only raises intriguing zoonotic implications (24) but also affords an opportunity to explore the pathologic effect of this human pathogen on feline gastric tissue. The goal of the study described here was to fully characterize *H. pylori* strains from cats and the histopathologic changes associated with *H. pylori* infection in domestic cats and to compare these findings with those noted in human gastric tissue infected with *H. pylori*.

MATERIALS AND METHODS

Animals. Gastric tissue was collected at necropsy from young adult cats from two different commercial vendors (vendors A and B) of research animals. Three

* Corresponding author. Mailing address: Division of Comparative Medicine, Massachusetts Institute of Technology, 45-104, 37 Vassar Street, Cambridge, MA 02139. Phone: (617) 253-1757. Fax: (617) 252-1877.

† Present address: Laboratory Animal Resources, Merck Research Laboratories, West Point, PA 19486.

‡ Present address: Department of Pathology, San Diego Zoo, San Diego, CA 92112.

cats, obtained from vendor A (whose cats were previously found to be free of *H. pylori* and GHLOs), were used as controls. Twenty-four cats were obtained from vendor B, whose cats we have documented to be infected with *H. pylori* (24). Vendors A and B both maintain purpose-bred, closed colonies of cats in which introduction of new animals occurs only rarely. Approximately equal numbers of males and females made up the study group.

The gastric tissue was harvested from cats being used in terminal procedures as part of various research projects. Six vendor B cats were present in the vivarium for 3 to 10 days and were used for acute studies of the respiratory tract. The remaining 18 vendor B cats and all 3 vendor A cats were used in chronic neurologic studies, and their stays in the facility varied from 3 to 7 months.

After arrival at our research facility, all cats were individually housed for the duration of their stay. Water was provided ad libitum in bowls, and the animals were fed laboratory feline diet 5003 (PMI Feeds, Inc., St. Louis, Mo.). None of the animals exhibited vomiting or other clinical signs referable to upper gastrointestinal disease. No oral medications were administered, nor were gastric intubation, gastroscopy, or any other procedures involving the digestive tract performed on any of the cats.

Isolation of gastric organisms. Four to five mucosal samples (approximately 3 by 3 mm) from both the body and the antrum of the stomach were collected for bacterial culture from 11 vendor B animals. The culture methods used have been described previously (24). Briefly, samples were ground and placed on a *Brucella* agar base supplemented with 5% sheep blood and containing trimethoprim, vancomycin, and polymyxin B (*Brucella* TVP agar; Remel, Lenexa, Kans.) and a chocolate agar base (Chocolate agar; Remel). Culture plates were incubated for 3 to 5 days at 35 to 37°C in a moist microaerophilic atmosphere provided by either an anaerobic jar with a Campybak system (Campybak Plus; Becton Dickinson Microbiology Systems, Cockeysville, Md.) or a sealed pouch (Biobag Environmental Chamber Type Cfi; Becton Dickinson Microbiology Systems). *H. pylori* organisms were identified as gram-negative, curved to slightly spiral rods and were further classified by biochemical testing as described previously (24).

Synthetic oligonucleotides and PCR amplification. Oligonucleotide primers were supplied by Bio-Synthesis, Inc. (Cambridge, Mass.). Primer pairs designated HpUI1-HpUI2 and HpU25F-HpU50F were designed to the urease A and B structural genes, respectively. Primer HpUI1 (5'-GCCAATGGTAAATT AGTT-3') and HpUI2 (5'-CTCCTTAATTGTTTTACATAGTT-3') amplified a 411-bp product from the *ureA* gene (nucleotide coordinates 304 to 714). Primers HpU25F (5'-CCCACACGGCCCCATCGCTT-3') and HpU50F (5'-GGCCCTACTACAGGCGAT-3') amplified a 1,070-bp product from the *ureB* gene (nucleotide coordinates 765 to 1835).

PCR amplification was performed in 100- μ l reaction volumes containing 10 mM Tris hydrochloride (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 μ M (each) the four deoxynucleoside triphosphates, 1 μ M (each) primer, and 2.5 U of *Taq* DNA polymerase (Promega). Approximately 200 ng of DNA from *H. pylori* ATCC 43504, *H. felis* CS1, and *H. pylori* isolated from two domestic cats was added as a template for each reaction. Reaction mixtures were overlaid with mineral oil to prevent evaporation, and after 4 min at 96°C the mixtures were subjected to 30 cycles of amplification in a programmable thermal cycler (Perkin-Elmer Cetus Corp.). Denaturation steps were carried out at 94°C for 30 s, the annealing step was carried out at 40°C for 30 s, and the extension step was carried out at 72°C for 1.5 min. At the end of the last cycle, samples were extended for a further 5 min at 72°C and were then cooled to room temperature. Twenty microliters of each sample was loaded onto a 1% agarose gel containing 1 μ g of ethidium bromide per ml. Control samples without DNA were run with each PCR.

Enzymatic digestion of amplified DNA. The amplified products obtained by PCR were subjected to restriction endonuclease digestion for 4 h at 37°C in 20- μ l volumes, as recommended by the manufacturer. The digested samples were analyzed by agarose gel electrophoresis (2% [wt/vol]). Restriction enzymes were chosen on the basis of the sequence data available for these amplified products.

Histologic evaluation. The stomach from each cat was opened along the lesser curvature, inspected for gross lesions, and then placed in 10% neutral buffered formalin. Full-thickness sections of gastric tissue approximately 1.5 cm in length from the cardia and fundus, body, and pyloric antrum of each cat were embedded in paraffin, cut into 5- μ m sections, and stained with hematoxylin and eosin and Warthin-Starry silver stains. For two vendor B animals, sections of esophagus and duodenum were also processed and stained with Warthin-Starry silver stain.

Warthin-Starry silver-stained sections were evaluated, on a blind-coded basis, for the presence of organisms and, if they were present, the morphologies of the organisms. The quantity of organisms seen in each 1.5-cm stained tissue section was graded by using the following scale: 0, no organisms seen; 1, few organisms (<10 organisms per section); 2, moderate numbers of organisms (10 to 50 organisms per section); 3, large numbers of organisms (>50 organisms per section, usually too numerous to count).

Hematoxylin and eosin-stained slides, also evaluated on a blind-coded basis, were assigned a gastritis score according to the following criteria: 0, normal; 0 to 10, inflammatory cells (not including those within lymphoid aggregates) per high-power field, with no lymphoid follicular aggregates and normal mucosal epithelium; 1, mild gastritis, 10 to 50 inflammatory cells per high-power field, fewer than two follicles per low-power field, and normal mucosal epithelium; 2, moderate gastritis, 10 to 50 or more inflammatory cells per high-power field, with greater than two follicles per low-power field and mild gastric epithelial changes;

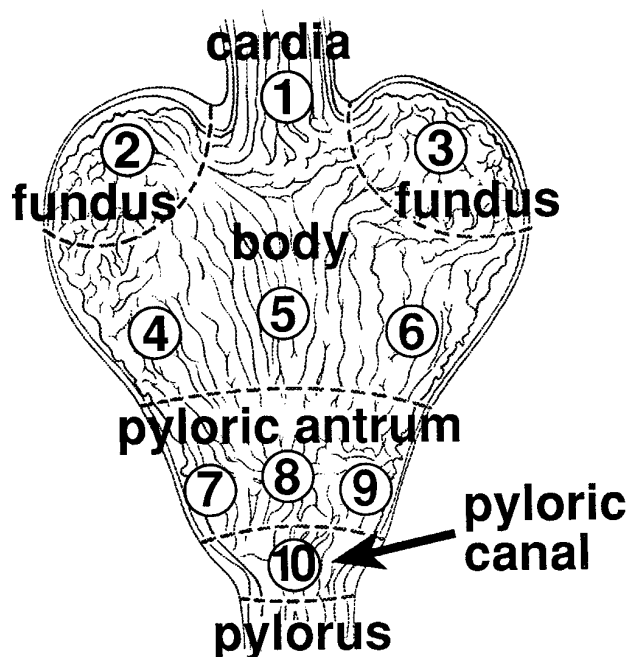


FIG. 1. Schematic of gastric biopsy sites for urease mapping in opened cat stomach (see Table 3). The figure is modified from reference 40.

3, severe gastritis, greater than 50 inflammatory cells per high-power field and marked epithelial changes. Epithelial changes included individual cell necrosis, cytoplasmic basophilia, and glandular dilation. The high-power field had a total magnification of $\times 400$, and the low-power field had a total magnification of $\times 20$. The type and location of inflammatory cells and the number of lymphoid follicles per low-power field were noted, as were spiral organisms. The average gastritis scores and the numbers of lymphoid follicles present for cats from vendors A and B were compared.

Electron microscopy. Tissue samples of gastric mucosa from a vendor B cat were fixed in 2.5% glutaraldehyde fixative, washed with cacodylate buffer, post-fixed with osmium tetroxide, dehydrated through graded acetone, and embedded in a Eponalrodite 6005 mixture (40). The plastic blocks were cut to a thickness of 1 μ m and were stained with methylene blue-azure II. The areas of interest were identified by light microscopy, cut from the plastic block, and mounted onto a plastic bean capsule. A 50- to 70-nm-thick section was then cut, stained with uranyl acetate-lead citrate, and visualized with a transmission electron microscope.

Urease mapping. Urease mapping was performed on seven vendor B cats to determine semiquantitatively the density of colonization by *H. pylori* in different regions of the stomach (28). One sample was taken from each of the cardia and the pyloric canal, two samples were collected from the fundus, and three samples were taken from both the stomach body and the antrum (Fig. 1). The areas of interest were placed into a rapid urease tube (Selective rapid urea; Remel). The tubes were incubated at room temperature, monitored hourly for 8 h, and evaluated for a final time at 24 h. The development of a pink color in the gel was considered a positive test result.

Statistics. Data were analyzed by nonparametric statistics. For comparison of gastritis scores and numbers of follicles, which were variables with few distinct scores, Cochran-Mantel-Haenszel statistics were used (43). Since sample sizes were not large enough to ensure the accuracies of asymptotic *P* values, exact permutation distributions were used (1). All *P* values were two sided. Results were considered statistically significant at *P* < 0.05.

Nucleotide sequence accession number. The sequences of the oligonucleotide primers are deposited in the EMBL Data Library under accession number X17079.

RESULTS

Isolation of *H. pylori*. Clear, pinpoint colonies were observed on culture of gastric antral samples from all 11 vendor B cats on which culture was attempted. Because of the failure of the microaerophilic environment, two of these cultures were subsequently lost and could not be characterized. Culture of bi-

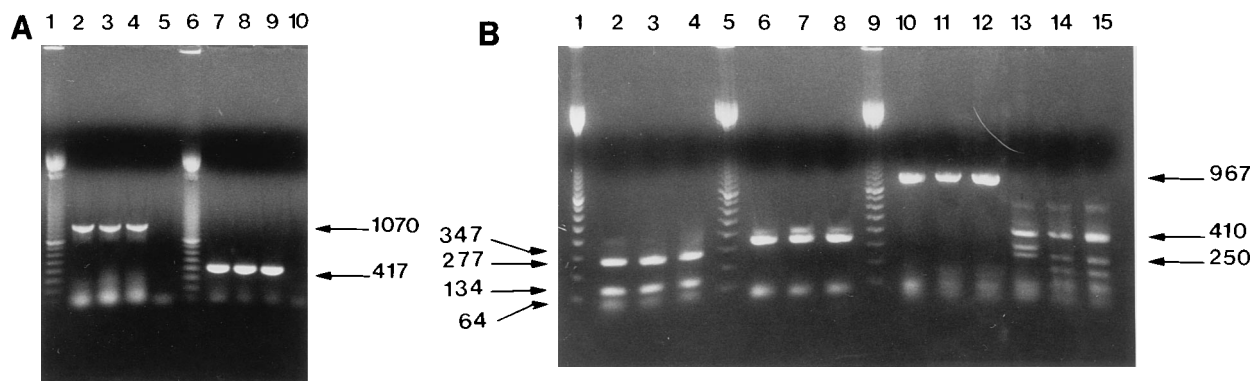


FIG. 2. (A) Gel depicting PCR products of *ureA* (411 bp) and *ureB* (1,070 bp) in *H. pylori* isolates from both cats and humans. (B) Gel depicting digestion of *ureA* with *HinfI* resulting in two fragments, 277 and 134 bp, conserved in isolates from both cats and humans (lanes 2 to 4). Digestion with *AhaI* gives 347- and 64-bp products; *Sau3AI* digestion of *ureB* identifies a polymorphism with the 1,070-bp gene fragment of the human and cat isolates (lanes 13 to 15).

opsy specimens from the gastric body yielded similar bacterial colonies from five animals.

The organisms obtained in cultures of samples from nine cats were identified as *H. pylori* on the basis of their appearance as gram-negative, curved rods measuring 3 to 5 μm in length and 0.5 μm in width and results from the following biochemical tests: positive for catalase, oxidase, urease, and alkaline phosphatase and negative for nitrate reduction, H_2S production in triple sugar iron agar, hippurate hydrolysis, and indoxyl acetate hydrolysis. The bacteria failed to grow at 25 and 42°C in a microaerophilic environment and at 37°C in an

aerobic environment and were resistant to nalidixic acid and susceptible to cephalothin.

Amplification of urease gene sequences from the *H. pylori* isolate from a domestic cat. By using *H. pylori* urease-specific gene primers designed to the DNA sequence from a clinical isolate from a human, it was possible to amplify both *ureA* and *ureB* homologs from two *H. pylori* isolates from domestic cats. Primer pairs specific for the *ureA* and *ureB* genes amplified a 411-bp product and a 1,070-bp product, respectively (Fig. 2A). Digestion of the 411-bp *ureA* product with the restriction enzyme *HinfI* resulted in two fragments of 277 and 134 bp; these

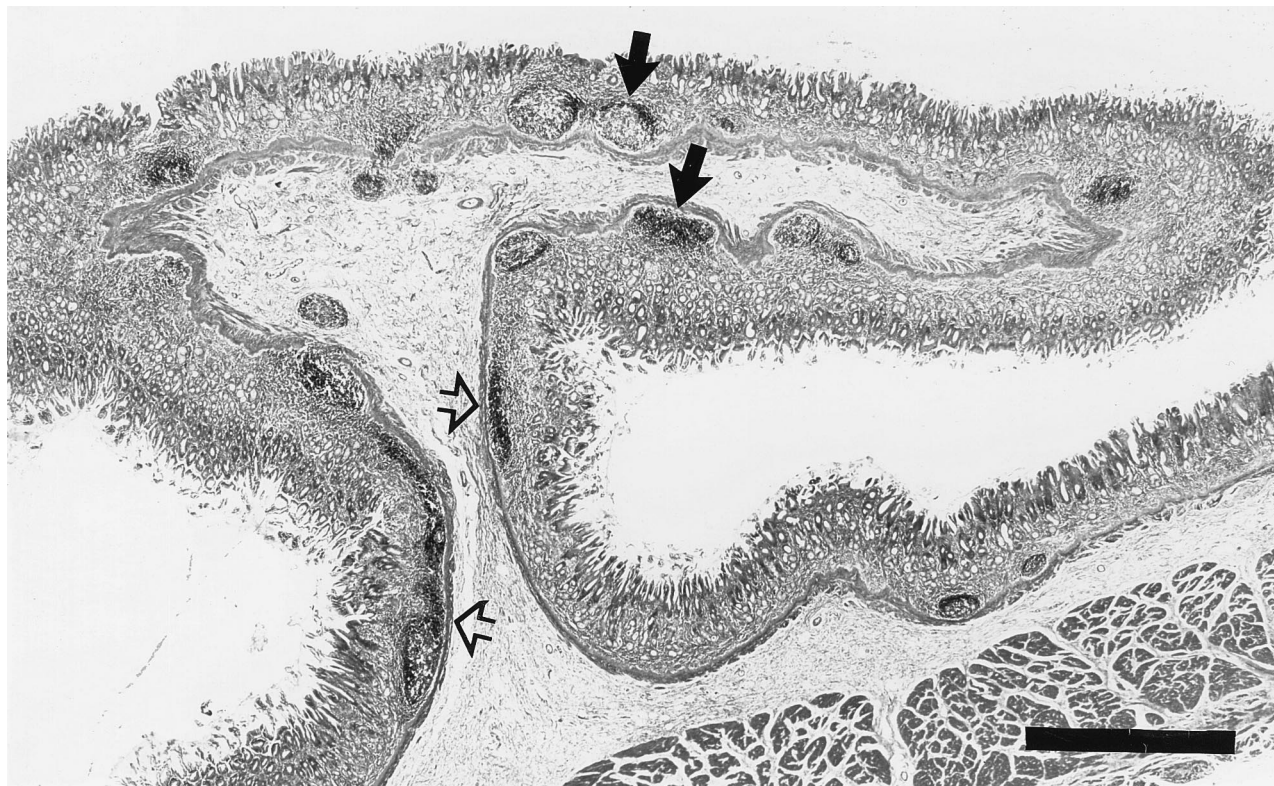


FIG. 3. Extensive infiltration of lymphoid cells (open arrows) with formation of lymphoid follicles (solid arrows) in the mucosa and submucosa of cat stomach. Hematoxylin-eosin stain was used. Bar, 1.13 mm.

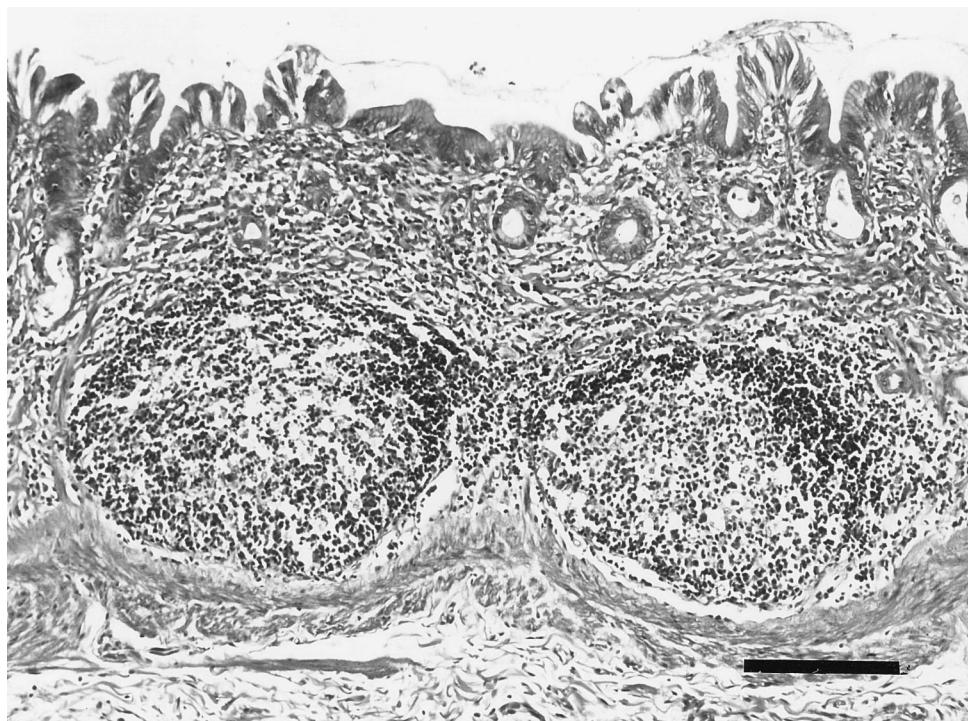


FIG. 4. Higher magnification of Fig. 2 (top middle) shows a marked effacement of the mucosal architecture by the lymphoid cells and lymphoid follicles in the mucosa of the cat stomach. Hematoxylin-eosin stain was used. Bar, 216 μ m.

fragments were conserved in the *H. pylori* isolates from both humans and cats (Fig. 2B, lanes 2 to 4). Digestion with the enzyme *AluI* gave rise to 347- and 64-bp products, and the products were equally conserved. Both profiles were characteristic of those from similar restriction fragment length polymorphism analyses performed elsewhere (7).

Restriction of the 1,070-bp *ureB* product with *HinfI* further confirmed urease gene sequence conservation; digestion with this enzyme gave rise to a 967-bp product and fragments that were beyond the resolution of the agarose gel (Fig. 2B, lanes 10 to 12). *Sau3AI*-digested *ureB* identified polymorphisms within the 1,070-bp gene fragment between the isolates from humans and cats (Fig. 2B, lanes 13 to 15). Two fragments with molecular sizes of 410 and 250 bp were conserved between isolates from both humans and cats, which is characteristic of what had been reported previously (8). Although differences exist between isolates from cats and humans, the *Sau3AI* profiles of isolates from cats were identical not only to each other but to those of *H. pylori* fingerprints of other isolates from humans reported previously (8). This provides some evidence of the conservation of these gene fragments between *H. pylori* strains isolated from different animal species but, as expected, highlights the existence of polymorphisms between strains. Fingerprinting patterns are now being determined for all isolates from cats from the same vendor to determine the clonal nature of the infecting strains.

Gross pathology and histopathology. Gross lesions were not observed in the stomach of any animal. Histologically, one vendor A cat had a gastritis score of 1 in the stomach body (fundic mucosa), and another cat had a gastritis score of 1 in the antrum. Inflammatory cells consisted of lymphocytes and plasma cells, predominantly in the deep mucosa. One cat also had occasional neutrophils, and small numbers of eosinophils were present in two cats. All other sections from vendor A cats

were normal (score of 0). No lymphoid aggregates or gastric spiral organisms were observed in sections from any of the three vendor A animals.

Seven of 24 vendor B cats had gastritis scores of 0 in the cardia and fundus, while 12 cats had scores of 1 and 5 were assigned a score of 2. Cardia was not identified histologically in 11 cats. Thirteen of 24 vendor B cats had gastritis scores of 0, whereas 9 vendor B cats had gastritis scores of 1 and 2 vendor B cats had scores of 2 in the stomach body (fundic mucosa). Three, 19, and 2 vendor B animals had gastritis scores of 1, 2, and 3, respectively, in the antrum. Twenty-one of 24 (88%) vendor B cats had gastritis scores of at least 2 in one region of the stomach, whereas this was the case for none of the 3 vendor A cats. The average gastritis scores for the cardia and fundus, body, antrum, and stomach as a whole were 0, 0.33, 0.33, and 0.67 and 0.92, 0.54, 1.96, and 1.96 for vendor A and vendor B cats, respectively. The scores for the antrum ($P < 0.001$) and stomach as a whole ($P < 0.01$) were significantly higher for vendor B cats than for vendor A cats.

The gastritis in *H. pylori*-infected cats (vendor B) consisted mainly of multifocal lymphoplasmacytic follicular aggregates in the deep mucosa, with the larger follicles displacing gastric glands as they approached the superficial mucosa (Fig. 3 and 4). Lymphocytes and plasma cells were the predominant inflammatory cells noted, while occasional neutrophils were present in three cats. Eosinophils were noted in small numbers in eight cats and in moderate numbers in two cats. Inflammatory cells were located mostly within the deep mucosa, but they were seen more superficially in the more severely affected animals. Twelve of 24 vendor B cats had lymphoid follicles in the cardia and fundus, and 10 had follicles in the stomach body (Fig. 4). All 24 vendor B cats had lymphoid follicles in the antrum, with an average of 6.4 follicles per section of antrum examined. The average number of follicles seen per section in

TABLE 1. Distribution of gastric lymphoid follicles by anatomic site in cats infected with *H. pylori*

Site	No. of cats with follicles (%) ^a	Average no. of follicles/section ^a	Total no. of follicles in sections ^a
Cardia or fundus	12 (50)	1.2	28
Body	10 (42)	0.9	22
Antrum	24 (100)	6.4	153
Total	24 (100)	8.5	203

^a Calculations from a study with a total of 24 cats. Sections were magnified 20×.

all three regions of the stomach combined for vendor B cats was 8.5; that for vendor A cats was 0. The numbers of lymphoid follicles in the antrum and in the stomach as a whole were significantly higher for vendor B cats than for vendor A cats ($P < 0.001$) (Tables 1 and 2).

In all 24 vendor B cats, histologic evaluation of Warthin-Starry silver-stained sections revealed the presence of comma- to spiral-shaped organisms (3 to 5 by 0.5 μm) overlying the gastric mucosa. The organisms were located in close proximity to the mucosal epithelial cells, in the lumina of the gastric pits, and in the superficial mucous layer (Fig. 5). In most of the cats, the bacteria were seen in large numbers in the cardia and fundus, body, and antrum. Twenty-three of the 24 cats had a 3+ quantity of organisms in at least one of the three areas of the stomach examined. The remaining cat had only a 1+ quantity of organisms in all three stomach regions. Seventeen of 24 cats examined had 3+ scores in all three regions of their stomachs. No organisms consistent in appearance with *H. pylori* could be found in the sections of esophagus or duodenum examined in two vendor B animals, although large numbers of *H. pylori* were present in the stomachs of these cats. *H. pylori* organisms were detected in only 6 of the 24 vendor B cats on evaluation of hematoxylin-eosin-stained sections.

Electron microscopy. The patterns of tissue reaction and the distribution of the bacteria were similar to those noted by light microscopy. Bacteria with typical *H. pylori* morphology and approximately 2.5 to 5 μm in length and 0.5 to 0.7 μm in width were present on the mucosal surfaces; they were often mixed with mucous strands or were found within the glandular lumina (Fig. 6) (55). Many of the bacteria were in intimate contact with epithelial cells. Three adherence patterns were commonly observed. First, the bacteria frequently adhered to microvilli of the epithelial cells, with membrane of glycocalyx fusion or formation of pedestal structures between the bacteria and microvilli (Fig. 7). Second, the epithelial cells were sometimes indented, with the bacteria adhering to the indented pits. Third, the bacteria were closely adhered to the epithelial cell surfaces, with or without disruption of the microvilli or being surrounded by the microvilli. Morphologic evidence of bacterial replication, i.e., binary fission, also was occasionally observed. Bacteria were not observed intracellularly.

Urease mapping. When the results for the seven vendor B cats on which urease mapping was performed are combined, six of the seven samples from the cardia became positive within 2 h and the other sample was positive by 8 h (Table 3). Eleven of the 14 fundic samples turned positive within 2 h, while the remaining 3 samples were positive by 8 h. Eleven of 21 samples from the stomach body were positive within 2 h, while 5 more samples were positive by 8 h, 3 were positive by 24 h, and 2 were negative. Fifteen of 21 antral samples were positive by 2 h, 4 more were positive by 8 h, and the remaining 2 were positive at 24 h. Five of the seven pyloric samples were positive by 2 h, while the remaining two became positive by 8 h. The

TABLE 2. Number of cats with gastric lymphoid follicles and total number of follicles in cats from two commercial vendors

Vendor	<i>H. pylori</i>	No. of cats	No. (%) of cats with follicles	Total no. of follicles in sections
A	Negative	3	0 (0)	0
B	Positive	24	24 (100)	203

short time interval (less than 1 h) in which many of the samples became positive is indicative of heavy colonization in many of the cats.

DISCUSSION

With the recent isolation of *H. pylori* from a group of domestic felines (24), three types of gastric spiral organisms have now been described in the domestic cat. *H. felis*, which has been isolated on artificial medium, is a large spiral bacterium characterized by periplasmic fibers (39). A second helical organism, not yet cultivated, is similar in appearance to *H. felis* but lacks fibers (39). This organism has been called the *Gastrospirillum*-like organism. On the basis of 16S rRNA analysis, the name "*Helicobacter heilmannii*" has recently been proposed for organisms with similar morphologies recovered from human stomachs (55).

We had previously shown, from sequencing a 1.5-kb fragment of the 16S rRNA gene, that the isolate from a cat shared 99.7% sequence identity with the human type strain (24). The sequence of the *H. pylori* strain from the cat differed by 5 bases (of 1,475 bases analyzed), typical of the minor variation seen in other *H. pylori* strains that have been sequenced (13). In an attempt to further characterize the *H. pylori* isolates from cats, genotypic studies were performed on strains isolated from dif-

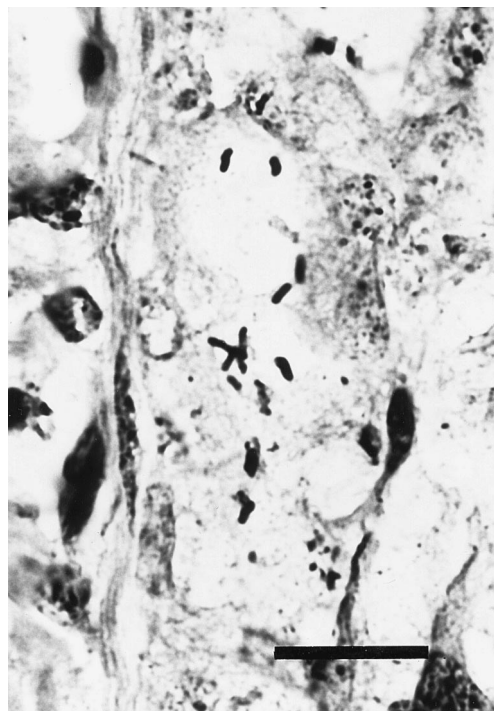


FIG. 5. Warthin-Starry silver stain revealed numerous *H. pylori* organisms on the gastric epithelium of a glandular crypt lumen of the cat stomach. Bar, 19.5 μm .

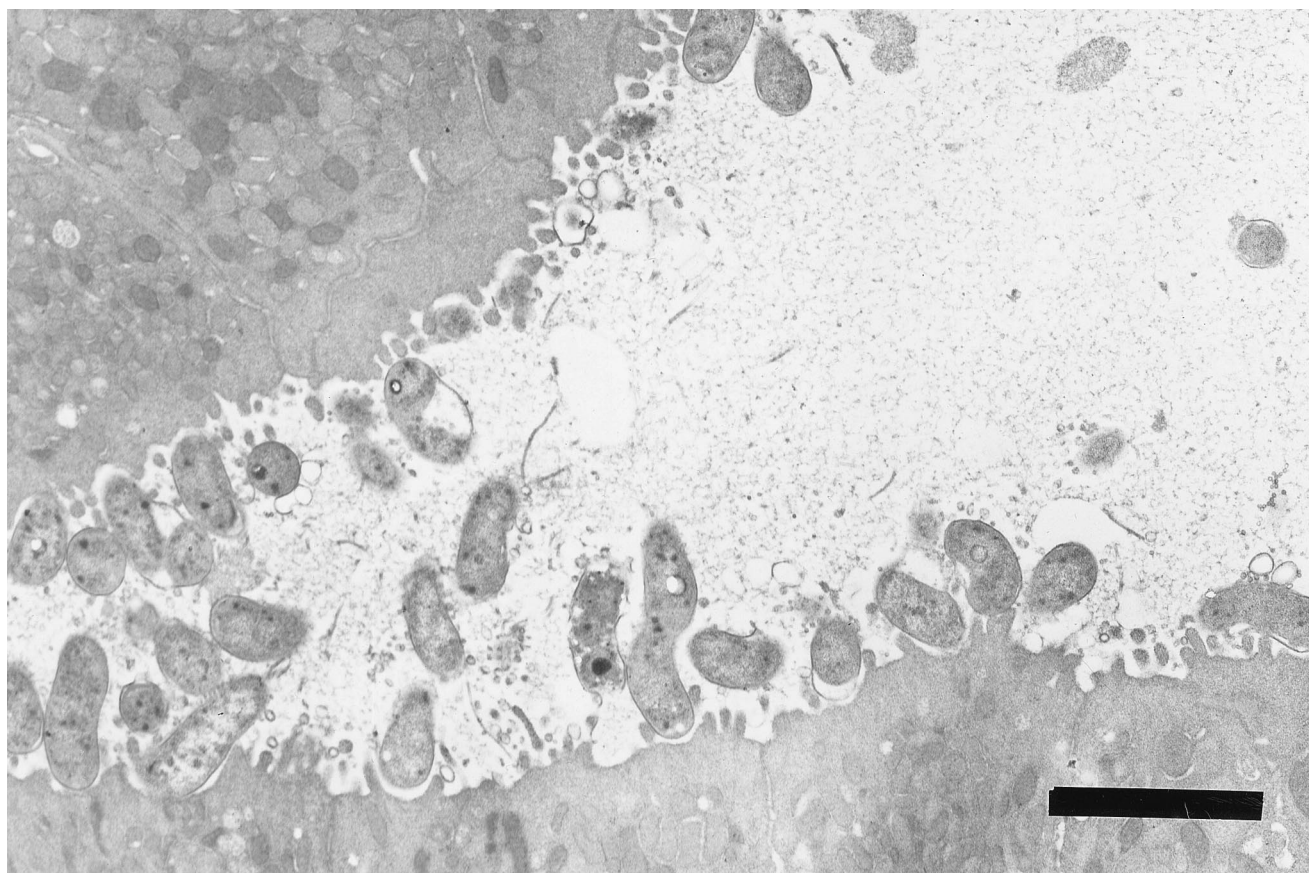


FIG. 6. Electron micrograph shows numerous *H. pylori* organisms attaching or adhering to the mucosal epithelial cell surfaces of glandular lumen of an infected cat stomach. Bar, 1.1 μ m.

ferent cats, and the genotypes were compared with those of the type strain from humans. Using gene-specific primers to the *ureA* and *ureB* genes of *H. pylori* from humans, we were able to amplify products of similar sizes from both studies of isolates from cats. Restriction fragment length polymorphism analysis of these gene fragments confirmed the presence of conserved regions compared with the sequence of the type strain from humans. The isolates from cats appeared clonal and showed little variation. Their isolation from cats acquired from the same vendor may reflect infection from a common source. Further genotypic studies carried out with primers derived from other known genes homogeneous to *H. pylori* and which are of human origin all amplify products of similar sizes from *H. pylori* isolates from both cats and humans and have confirmed the strains' similarities by antigenic relatedness (results will be reported elsewhere).

The results of urease mapping performed on *H. pylori*-infected cats indicated that colonization was less dense in the body of the stomach compared with the density in the cardia and fundus, antrum, and pylorus. However, even in the stomach body, *H. pylori* organisms were abundant in most cats on the basis of an evaluation of histologic sections. In contrast, GHLOs have been reported to be more prevalent in the fundus and body of the stomach than in the antrum and pylorus (48). GHLOs were not observed in the *H. pylori*-infected cats, indicating that the cats, which originated from a closed colony, were probably not exposed to these organisms. Competitive exclusion of GHLOs by *H. pylori* in the cat is a possibility; in humans, dual infection with *H. pylori* and GHLOs is rare, and

competition between the organisms has been postulated (35). However, *H. pylori* and organisms similar in appearance to GHLOs have been found to coexist in the stomachs of rhesus monkeys (52).

H. pylori infection was associated with a lymphofollicular gastritis in the colony of domestic cats described here. The infection was characterized by lymphofollicular aggregates in the deep mucosa. Lymphoplasmacytic infiltrates in the superficial mucosa, as well as neutrophils and eosinophils, were noted in some animals. In most cases, the inflammatory infiltrate was not accompanied by epithelial changes. Eighty-eight percent of the *H. pylori*-infected cats had at least a moderate lymphofollicular gastritis in one region of the stomach, with the majority of the inflammation occurring in the gastric antrum. In contrast, none of the vendor A cats, which lacked organisms, had even a moderate gastritis. The lymphofollicular gastritis noted in *H. pylori*-infected cats is similar to that observed in cats colonized by GHLOs. In one report, cats colonized by GHLOs were found to have multiple, subglandular lymphoid nodules and focal extensions of leukocytes from the subglandular region into the lamina propria and submucosa (48). The inflammatory infiltrates in the cats were mainly composed of lymphocytes and plasma cells, but they occasionally contained neutrophils and eosinophils (48).

In contrast to the *H. pylori*-infected cats, most humans colonized by *H. pylori* have an active component to their gastritis, typically characterized by neutrophils and glandular microabscesses, as well as lymphocytes and plasma cells (37). However, gastric lymphoid follicles are commonly observed in adult hu-



FIG. 7. Higher magnification of Fig. 6 (bottom left) shows a close association of *H. pylori* with the epithelial cells of the cat stomach. Bar, 400 nm.

mans infected with *H. pylori* (2, 20, 56) and are the predominant lesion noted in children infected with the organism (25). Several species of nonhuman primates develop chronic gastritis when they are naturally or experimentally infected with *H. pylori* (3, 26, 46). This gastritis in the *H. pylori*-infected primates tends to be more diffuse than that in the *H. pylori*-infected cats (46).

Experimental infection with *H. pylori* causes chronic gastritis in nude mice (30) and lymphofollicular gastritis in gnotobiotic pigs (33). Gnotobiotic beagle dogs have also been successfully infected with *H. pylori* (51). These animals developed focal to

diffuse lymphoplasmacytic infiltrates with follicle formation, especially in the antrum, and mild to moderate infiltration of neutrophils and eosinophils in the gastric lamina propria (51). This chronic active gastritis resembled that seen in infected humans; however, as with the gnotobiotic pigs (33), the study was limited to 30 days (51). We have recently experimentally infected naive specific-pathogen-free cats with an *H. pylori* strain from a cat. All cats became persistently colonized as determined by gastric culture and PCR during serial gastric biopsies and necropsy at 7 months postinfection (15). All cats experimentally infected with *H. pylori* had multifocal gastritis consisting of lymphoid aggregates plus multiple large lymphoid nodules, which were most noticeable in the antral mucosa. In addition, one *H. pylori*-infected cat had a moderate diffuse infiltration of polymorphonuclear leukocytes in the subglandular region of the antrum. *H. pylori*-like organisms were focally distributed in glandular crypts of the antrum. Selected *H. pylori*-infected cats had a significant eightfold increase in the level of immunoglobulin G antibody to *H. pylori* in serum over the baseline level. In contrast to the *H. pylori*-infected cats, urease mapping indicated that *H. pylori* colonization in the dogs was heaviest in the fundus (body) of the stomach rather than in the cardia or antrum (51). Also, gastric biopsy samples from the dogs required several hours to turn the urease assay positive (51), whereas gastric biopsy samples from the cats often turned the urease test positive in less than an hour, suggesting that *H. pylori* colonization in the naturally infected cats was heavier than that in the experimentally infected dogs.

TABLE 3. Results of urease mapping of the stomach of domestic cats infected with *H. pylori*

Cat no.	Biopsy site for urease map ^a									
	1	2	3	4	5	6	7	8	9	10
1	+++	+++	++	+	0	0	+++	+++	+++	+++
2	+++	+++	+++	+++	++	++	+++	+++	+++	+++
3	+++	+++	++	+++	+++	+	++	+++	++	++
4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
5	+++	+++	+++	++	+	+++	+++	+	+++	+++
6	++	+++	++	+++	++	++	++	+	++	++
7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

^a Sites: 1, cardia; 2 and 3, fundus; 4 to 6, body; 7 to 9, antrum; 10, pylorus (see Fig. 1). Urease scores were based on the following criteria: 0, no reaction within 24 h; +, positive reaction at 8 to 24 h; ++, positive reaction at 2 to 8 h; +++, positive reaction at <2 h.

The small to moderate numbers of eosinophils noted in the gastric mucosae of several cats has also been noted in the stomachs of cats colonized with *H. felis* and other GHLOs (48) and gnotobiotic dogs experimentally infected with *H. pylori* (51). Eosinophils have been found to be a component of the inflammatory response to helicobacters in some humans (19, 44, 53) and animals (18, 18a, 38, 40, 51), particularly during the acute phase of infection. A recent study in humans found increased eosinophil infiltration and degranulation associated with chronic gastritis caused by *H. pylori* (44). Thus, bacterial infection may be a stimulus for eosinophilic infiltration in the gastric mucosae of animals and humans.

The presence of lymphoid follicles has traditionally been considered a common, nonspecific finding in the gastric mucosae of dogs and cats. However, in the present study the cats free of gastric organisms showed a complete absence of lymphoid follicles. In contrast, *H. pylori*-infected cats had large numbers of follicles, especially in the antrum, and cats harboring *H. felis* or other GHLOs have also been documented to form follicles (48). In another study, gnotobiotic dogs experimentally infected with *H. felis* developed large numbers of lymphoid follicles throughout the gastric mucosa, while two control animals had no follicles (40). Our data and the high prevalence of lymphoid follicles associated with GHLO infection in cats and dogs indicate that lymphoid follicles found in the gastric mucosae of small animals are in most cases formed in response to stimulation by GHLOs. Similarly, recent studies indicated that lymphoid follicles were absent from the stomachs of healthy humans, whereas gastric lymphoid follicles have been found in a large percentage of patients with *H. pylori* infection (20, 56); in one report gastric lymphoid follicles were observed in 100% of the *H. pylori*-positive humans studied (56). The follicles are often more numerous in the antrum than in the more proximal areas of the stomach (56). These data, plus the fact that *H. pylori* infection in children frequently results in marked gastric lymphoid hyperplasia (25), have led to the hypothesis that lymphoid follicles in the human gastric mucosa can result from chronic *H. pylori* antigenic stimulation and, therefore, represent a specific immune response directed against the organism (20, 56). Also, by inducing lymphoid tissue formation in the gastric mucosa, *H. pylori* may be a necessary precursor for the development of primary gastric MALT lymphoma (9, 29, 61).

The ultrastructural morphologies of the *H. pylori* organisms examined in the present study are typical of those described previously for isolates from humans (6, 41). Ultrastructural studies of *Helicobacter* spp., including *H. pylori*, in humans and animal systems revealed a close association of the bacteria with gastric epithelial cells (5, 6, 41, 47, 54). Several adherence patterns have been identified, including formation of pedestals, indentation sites, and cup-like projections between the bacteria and epithelial cells. In the present study, fusion or pedestal formation between bacterial membranes and the epithelial microvilli and close opposition of the bacteria to epithelial cells at the indentation sites were frequently observed. These structures may be important for the signal transduction or material interchanges between the bacteria and the epithelial cells. Adhesion of the bacteria to gastric epithelial cells has commonly been observed with ulcerogenic *Helicobacter* spp., such as *H. pylori* and *H. mustelae*, but not with nonadherent *H. felis* and *H. heilmannii*, which induce gastritis without concurrent ulcer formation (6, 41, 47). Intracellular invasion of *H. pylori* and *H. heilmannii* into the parietal cells with cell degeneration has been infrequently documented in humans (5, 6, 27). However, intracellular invasion of *H. pylori* and epithelial cell degeneration were not observed in the study described here.

The *H. pylori*-infected cats did not have grossly evident lesions or clinical signs referable to gastritis. However, several recent reports indicate that GHLOs may be associated with clinically significant gastritis in felines. A group of nine Persian cats with severe chronic gastritis was observed to harbor gastric organisms consistent in appearance with *H. felis* and other GHLOs, and because of the absence of other potential etiologic agents, the investigators concluded that the GHLOs may have been responsible for the lesions (14). A group of cheetahs infected with *H. acinonyx* and GHLOs developed severe lymphoplasmacytic gastritis, which was associated with weight loss and gastric ulceration (12). Chronic gastritis, which is associated with occasional vomiting, is observed in dogs and cats, and an etiology for this condition is rarely diagnosed (58). The role of GHLOs in the development or potentiation of this gastritis must be considered.

A small number of humans with gastritis has been found to be colonized by organisms morphologically similar to *H. felis* or other GHLOs (27, 36, 45, 60). Indeed, gastritis induced by *H. felis* in a research worker was suspected to be a direct result of working with cat stomachs infected with this bacterium (34). Also, a 12-year-old girl was diagnosed with active chronic gastritis, believed to be due to infection with GHLOs acquired from a pet dog (57). These reports have led to speculation that these organisms may be zoonotic, with transmission occurring from dogs and cats to humans (34, 36, 39, 57, 60). The presence of *H. pylori* in domestic cats raises similar zoonotic concerns (24). Although water has been cited as a possible source of *H. pylori* (32), transmission of *H. pylori* has traditionally been considered to be primarily from person to person (10, 31), in part because no environmental reservoir of the organism had been conclusively identified. However, the potential of the domestic cat to act as an environmental reservoir for *H. pylori* introduces a new dimension into the epidemiology of *H. pylori* disease in humans and also raises the possibility that other domestic animals could be reservoirs of *H. pylori*.

In summary, *H. pylori*, along with GHLOs, is now linked with lymphofollicular gastritis in the domestic cat. Whether this *Helicobacter*-associated lymphofollicular gastritis leads to clinical signs will require further detailed studies. *H. pylori* has been cultured from saliva and gastric wash of additional cats from the same commercial source that were subsequently surveyed, and the presence of *H. pylori* in feces has been ascertained by PCR (18b). The presence of *H. pylori* at these sites increases the likelihood of zoonotic transmission because of human exposure to cat feces during routine cleaning of litter boxes and because of cats' propensity to vomit and, finally, because cats are fastidious groomers. Studies are ongoing to further define the prevalence and significance of *H. pylori* infection in the domestic cat.

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